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Microbial Decomposition of Methionine and Identity of the Resulting Sulfur Products[1]

WILLIAM SEGAL[2] AND ROBERT L. STARKEY

Department of Biochemistry and Microbiology, College of Agriculture and Environmental Science, Rutgers, The State University, New Brunswick, New Jersey 08903

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Various bacteria, actinomycetes, and filamentous fungi decomposed methionine, but only certain aerobic bacteria isolated from soil decomposed it in the absence of other organic substrates. These bacteria could grow on methionine as the only organic substrate and source of nitrogen and sulfur. Methionine was first deaminated and then demethiolated with production of methanethiol, part of which was oxidized to dimethyl disulfide. The amount of methanethiol that was oxidized varied with different cultures. A bacterial culture initially unable to grow on methionine developed capacity to do this in a medium which contained methionine and other growth substrates. The two sulfur products, methanethiol and dimethyl disulfide, are volatile and escaped from the media, resulting in a decrease in the sulfur content proportional to the amount of methionine decomposed.

There is a great diversity of organic sulfur compounds and they are fundamentally important biologically. Therefore, it is surprising that little is known about their decomposition—the microorganisms involved, the course of their dissimilation, and the products formed. One of the most significant organic sulfur compounds is methionine, which is attacked by certain bacteria and filamentous fungi with release of the nitrogen and sulfur (1, 2, 8, 17, 24–26). The sulfur product most frequently reported is methanethiol. Other products are dimethyl disulfide (4, 13, 26), dimethyl sulfide (4), ethyl sulfide (1), hydrogen sulfide (4, 23, 26), and sulfate (9, 23). In most reports of methionine decomposition, ability of the cultures to grow on the amino acid was not indicated. The cultures were grown in media which contained potential substrates in addition to methionine (1, 4, 9, 14, 23), or the methionine transformations were made by washed cells and cell-free extracts (2, 14–16, 18, 19, 27). Growth at the expense of methionine would be expected to result in oxidation of the carbon chain that remained after removal of the sulfur. This carbon chain has been reported to be α-keto butyric acid (1, 13, 15, 16, 20, 27).

We determined the ability of representative bacteria, actinomycetes, and filamentous fungi to attack methionine, and we isolated bacteria which grew on the amino acid. Principal attention was directed to establishing the sulfur products of the breakdown of methionine by two of these bacteria.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, New Brunswick.

[2] Present address: Department of Biology, University of Colorado, Boulder, Colo.

MATERIALS AND METHODS

Cultures and cultural methods. Stock cultures of 28 representative bacteria, actinomycetes, and fungi were cultivated on agar slants containing mineral salts, 0.05% NH_4NO_3, 0.1% peptone, 0.1% glucose, and 0.5% methionine. They were then tested for ability to decompose methionine. Other bacteria and streptomycetes were isolated from soil on plates of methionine agar. The following basal medium was used in which methionine was the only organic compound and source of nitrogen and sulfur: K_2HPO_4, 2.0 g; KH_2PO_4, 1.0 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $MgCl_2 \cdot 6H_2O$, 0.1 g; $FeCl_3 \cdot 6H_2O$, 0.01 g; and DL-methionine, 5.0 g. The volume was brought to 1 liter and the *p*H was adjusted to 7.0. Cultures were inoculated into 50- or 100-ml portions in 250-ml flasks and were shaken during incubation at 28 or 37 C, according to their optimal temperatures. In experiments with the bacteria which grew on methionine, the inoculum consisted of cells washed three times with neutral buffer and had an equivalent dry weight of 10 mg.

Analytical. Sulfate was determined gravimetrically as the barium salt. A qualitative test was made for polythionates (10). Total sulfur was determined by a method of Evans and St. John (6) modified to include a mixture of bromine and carbon tetrachloride in the digestion solution. Attached to the digestion flasks was a gooseneck condenser with bulbs containing iodic anhydride to trap volatile sulfur compounds (12, 22). Methionine was determined by two methods. One was the Lavine method (14), which depends on the pres-

ence of both the α-amino and methiol groups. The other method was that of McCarthy and Sullivan modified by Hess and Sullivan (11), referred to as the Sullivan method. This method is specific for the methiol group. Amino nitrogen was determined by Formol titration, sulfhydryl compounds were detected by sodium nitroprusside, and cysteine, by the method of Csonka et al. (5). To detect volatile sulfur products, sterile air was drawn through the culture solutions into 4% $Hg(CN)_2$ and 3% $HgCl_2$. Precipitates in the trap solutions were washed and recrystallized, and the compounds were identified by color, melting points, mixed melting points with known compounds, and reactions with alkali and ammonium thiocyanate (3, 21). Methanethiol, dimethyl disulfide, and dimethyl sulfide were determined quantitatively by the method of Segal and Starkey (21).

RESULTS

Transformation by representative stock cultures. Cultures of 12 bacteria, 6 actinomycetes, and 10 fungi from the stock collection were inoculated into flasks of the basal methionine medium and of a similar medium which contained glucose and peptone also. Loss of methionine was determined by the Lavine method after the cultures had incubated for 1 week. In the basal medium, none of the actinomycetes and fungi grew and decomposed methionine, but small amounts (2 to 6%) of the amino acid were broken down by 9 of the 12 bacteria. Most cultures attacked methionine in the medium that contained glucose and peptone. Results representative of the 28 cultures are included in Table 1. All but 6 of the cultures decomposed more than 10% of the amino acid, and 20 produced methanethiol. No dimethyl disulfide, dimethyl sulfide, cysteine, hydrogen sulfide, or sulfate was detected.

Decomposition by soil isolates. A cystine-decomposing bacterium (culture 14) isolated from soil failed to grow in the basal medium and failed to decompose methionine when first isolated; however, after repeated cultivation in a medium containing methionine, glucose, and peptone, it decomposed methionine in the basal medium as rapidly as any of the soil isolates. It grew poorly when transferred back to the cystine medium but grew normally on the second serial transfer. It lost no ability to attack methionine when it was cultivated in nutrient solution and in a cystine medium.

Many cultures of bacteria and streptomycetes were isolated from soil on methionine-agar. None of the streptomycetes grew well or decomposed much methionine in the basal medium. Several of the bacteria grew on methionine in the basal medium. They were gram-negative, nonspore-forming, motile rods that produced light greenish-orange iridescence on nutrient agar and formed convex, smooth, wet, gray colonies on methionine-agar.

TABLE 1. *Decomposition of methionine by some microorganisms*

Organism	Methionine decomposed[a] with methionine as substrate	Methionine, glucose, peptone as substrate	
		Methionine decomposed[a]	Methanethiol
	%	%	
Pseudomonas fluorescens	4	12	+
Proteus vulgaris	4	48	+
Sarcina lutea	2	4	+
Bacillus subtilis	0	27	0
Streptomyces griseus	0	54	+
S. lavendulae	0	17	+
Micromonospora sp.	0	4	+
Rhizopus nigricans	0	23	0
Aspergillus oryzae	0	48	+
Fusarium culmorum	0	62	+
Candida albicans	0	4	+

[a] Methionine was determined by the Lavine method.

Glucose increased greatly methionine decomposition by both streptomycetes and bacteria, but the percentage increase in decomposition was greater with the streptomycetes (Table 2). Small amounts of the amino acid were decomposed by the streptomycetes and the fungus in static or shaken basal medium (Table 3). Shaking promoted decomposition by the bacteria, but decomposition was rapid in the static medium.

Changes in sulfur content of media. Methionine disappeared without accumulation of sulfur products in the medium during bacterial decomposition. As the methionine content decreased, there was a smaller decrease in the amount of total sulfur (Table 4), which is ascribed to the method used to determine methionine. Since the Lavine method depends on the presence of both the amino and methiol groups, methionine that was deaminated but not demethiolated would not have been detected. The results are interpreted as reflecting greater deamination than demethiolation.

Volatile sulfur products. Methanethiol and dimethyl disulfide were identified as the only volatile sulfur products. During development of cultures for 3 days, quantitative determinations of these products were made according to methods A and B, previously described (21), in separate experiments.

The amounts of total sulfur and of methionine sulfur (determined by the Sullivan method) that remained in the culture solutions were the same

TABLE 2. *Influence of glucose on decomposition of methionine*[a]

Organism	Substrates		Growth[b]	pH	Methionine decomposed[c]
	Glucose	Methionine			
	%	%			%
Streptomyces sp. 8	1	0	++	4.4	
	0	0.5	+	6.9	8
	0.5	0.5	++	4.1	40
Streptomyces sp. 9	1	0	++	5.6	
	0	0.5	+	6.9	5
	0.5	0.5	++	5.6	25
Bacterium 10	1	0	+++	3.6	
	0	0.5	+++	8.1	30
	0.5	0.5	++++	4.0	60
Bacterium 11	1	0	+++	3.6	
	0	0.5	+++	8.2	40
	0.5	0.5	++++	4.1	65

[a] Incubation 13 days.
[b] Key: +, slight growth; ++, fair growth; +++, good growth; ++++, abundant growth.
[c] Methionine was determined by the Lavine method.

TABLE 3. *Effect of shaking on decomposition of methionine*

Organism	Condition of incubation	Methionine decomposed[a]		
		6 days	7 days	21 days
		%	%	%
Streptomyces sp. 8	Static		7	8
	Shaken		10	13
Streptomyces sp. 9	Static		8	9
	Shaken		12	13
Penicillium sp. 16	Static		4	6
	Shaken		9	11
Bacterium 10	Static	51	64	
	Shaken	83	99	
Bacterium 14	Static	62	98	
	Shaken	87	100	

[a] Methionine was determined by the Lavine method.

and are so indicated in Fig. 1. The possibility is not excluded that there was some α-keto-γ-methyl mercapto butyric acid, which is a deamination product of methionine. If any was present, it would have been determined as methionine by the Sullivan method. No other sulfur products accumulated in the medium; when the experiment was concluded all of the methionine sulfur had been recovered as the two volatile products, methanethiol and dimethyl disulfide. No dimethyl sulfide or inorganic sulfur compounds were detected. Most of the methionine sulfur was transformed to dimethyl disulfide by bacterium 12 (Fig. 1), but methanethiol was the first cleavage product. The amount of methanethiol was maximal in 16 hr; subsequently, all of the volatile sulfur was dimethyl disulfide. When all methionine had disappeared, 90% of the sulfur had been converted to dimethyl disulfide and 10% to methanethiol. Bacterium 14 transformed most of the methionine sulfur (90%) to methanethiol. The rest was dimethyl disulfide that was produced late in the incubation period.

The results suggested that the bacteria differed in capacity to oxidize the thiol to dimethyl disulfide. The following experiment provided additional information about the oxidation of methanethiol and related compounds by the two bacteria. Flasks containing measured quantities of methanethiol, dimethyl disulfide, and dimethyl sulfide dissolved in 100-ml portions of water were placed in absorption trains just ahead of flasks containing cells (equivalent to 15 mg, dry weight) of the bacterial cultures suspended in 100-ml amounts of a mineral salts solution. Vessels with reagents to trap volatile sulfur compounds followed the culture flasks. Determinations were made of the oxidation of the sulfur compounds that were drawn through the cell suspensions in an air stream during approximately 0.5 hr.

Methanethiol was oxidized to dimethyl disulfide by both bacteria, to a greater extent by bacterium 12 than by bacterium 14. Neither dimethyl disulfide nor dimethyl sulfide was altered by the bacteria; all was recovered in the trap solutions (Table 5).

Deamination and demethiolation of methionine. Since the results were the same for the two bacteria, only those for bacterium 14 are reported (Fig. 2). The courses of decrease in amino nitrogen and in methionine, determined by the Lavine method, were nearly identical. Deaminated but otherwise unaltered methionine would not be determined as methionine by this method, which depends on the presence of both the amino and methiol groups.

The course of loss of methionine determined by the Sullivan method, which is specific for the intact methiol group, was slower than deamination and followed the course of decrease in total sulfur in the culture solution and accumulation of volatile sulfur products. The difference between the upper and lower groups of curves probably reflects the amount of the deaminated product of methionine, α-keto-γ-methyl mercapto butyric acid. Its eventual disappearance is indicated by the meeting of the curves at 72 hr.

TABLE 4. *Sulfur content of medium during decomposition of methionine*[a]

Organism	Condition of incubation	4 days		7 days		10 days	
		Methionine S	Total S	Methionine S	Total S	Methionine S	Total S
		mg	*mg*	*mg*	*mg*	*mg*	*mg*
Bacterium 10	Static	25	37	0	2	0	2
	Shaken	34	42	19	23	1	7
Bacterium 14	Static	7	9	0	4	0	3
	Shaken	20	27	1	13	0	5

[a] Initial content of methionine S and total S in 50 ml of medium was 54 mg (1.684 mmoles). Methionine was determined by the Lavine method.

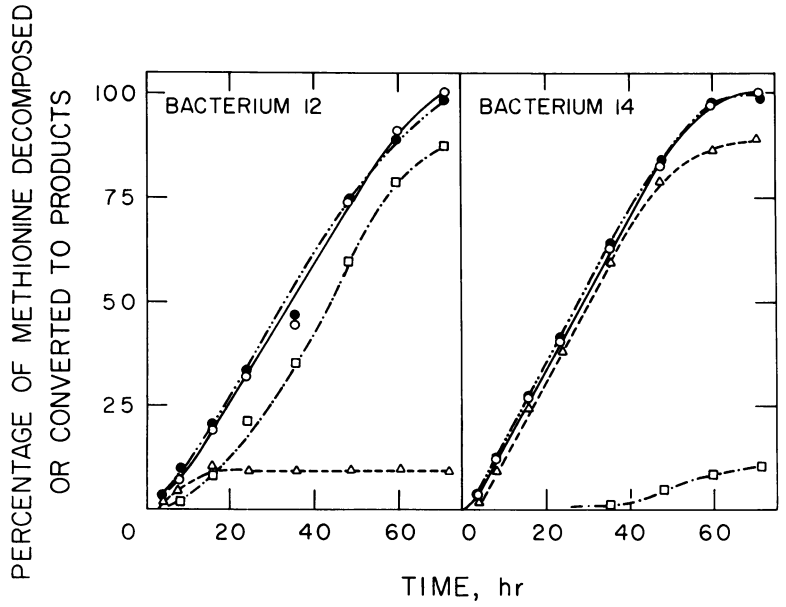

FIG. 1. *Products of decomposition of methionine. Decrease in methionine sulfur* (○) *and total sulfur* (●); *sulfur recovered as methanethiol* (△) *and dimethyl disulfide* (□).

DISCUSSION

The results establish that diverse bacteria, actinomycetes, and fungi attack methionine but that most of them require other organic compounds such as glucose to support growth. Ability to grow on methionine is a property of exceptional microorganisms. The cultures recovered from soil were bacteria of the genus *Pseudomonas*, *Achromobacter*, or *Flavobacterium*. Methanethiol and its oxidation product, dimethyl disulfide, were the only sulfur products detected, and they accounted for nearly 100% of the sulfur of the decomposed methionine. Challenger and Charlton (3, 4) reported that dimethyl sulfide was produced from methionine by *Scopulariopsis brevicaulis* through methylation of the product methanethiol. No dimethyl sulfide, ethyl sulfide, cysteine, sulfide, thiosulfate, polythionates, sulfite, or sulfate was detected as a product of methionine breakdown by the bacteria.

The bacteria first deaminated methionine and then demethiolated it with production of the volatile sulfur product methanethiol. A portion of the mercaptan was oxidized to dimethyl disulfide, and the capacity to effect this oxidation differed with the various cultures that were used. The amount of sulfur released from the methionine was equivalent to that of the volatile products. The fact that methionine was attacked by some microorganisms without production of methanethiol (Table 1) is ascribed to deamination of the amino acid without demethiolation. Loss of methionine as determined by the Lavine method and loss of amino nitrogen was more rapid than

TABLE 5. *Oxidation of sulfur compounds by methionine-decomposing bacteria*

Inoculum	Compound added		Percentage of the sulfur recovered as		
	Name	Amt (mmoles)	Methanethiol	Dimethyl disulfide	Dimethyl sulfide
Uninoculated	Methanethiol	1.862	97	2	0
Bacterium 12	Methanethiol	1.862	57	41	0
Bacterium 14	Methanethiol	1.862	80	19	0
Uninoculated	Dimethyl disulfide	1.123	0	98	0
Bacterium 12	Dimethyl disulfide	1.123	0	98	0
Bacterium 14	Dimethyl disulfide	1.123	0	98	0
Uninoculated	Dimethyl sulfide	1.363	0	0	98
Bacterium 12	Dimethyl sulfide	1.363	0	0	98
Bacterium 14	Dimethyl sulfide	1.363	0	0	97

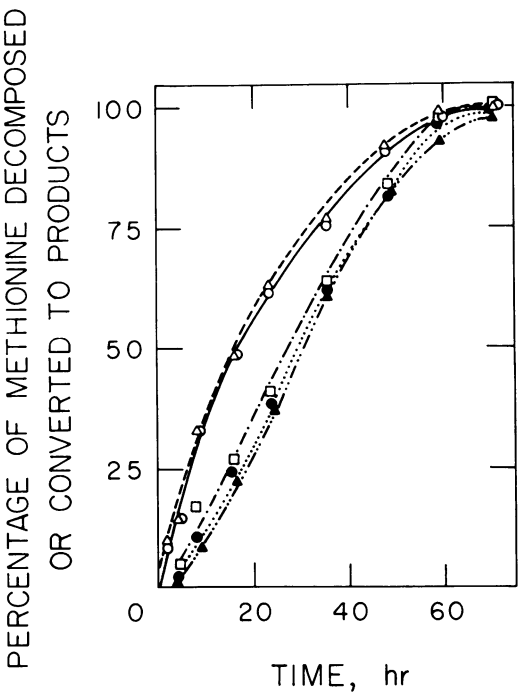

FIG. 2. *Course of deamination and demethiolation of methionine by bacterium 14. Decrease in methionne by Lavine method* (○), *amino nitrogen* (△), *total sulfur in medium* (□), *and methionine by Sullivan method* (●); *increase in volatile sulfur products* (▲).

loss of total sulfur from the culture solutions through volatilization of the sulfur products (Table 4, Fig. 2). This is attributed also to the production of deaminated methionine, α-keto-γ-methyl mercapto butyric acid, and its persistence for a short time before it was demethiolated. These results suggest that deamination preceded demethiolation.

One of the bacteria that did not decompose methionine initially decomposed it rapidly after prolonged cultivation in media containing the amino acid. It retained capacity to decompose methionine after cultivation on a medium lacking methionine. This suggests that a mutant was selected. During development on methionine, both cultures oxidized the demethiolation product, methanethiol, to dimethyl disulfide, but at different rates (Fig. 1). The fact that the ratio of dimethyl disulfide to methanethiol increased during decomposition suggests that the enzyme system involved in the oxidation of methanethiol is constitutive and became increasingly active in the presence of its substrate.

The results indicate that DL-methionine was decomposed as follows by the bacteria:

$$\mathrm{HOOC.CH(NH_2).CH_2.CH_2.S.CH_3}$$

Oxidative deamination ↓

$$\mathrm{HOOC.CO.CH_2.CH_2.S.CH_3 + NH_3}$$

Demethiolation ↓

$$\mathrm{HOOC.CO.CH_2.CH_3 + HS.CH_3}$$

$$\mathrm{CO_2 + H_2O} \qquad \mathrm{\tfrac{1}{2}(CH_3.S.S.CH_3)}$$

Kallio and Larson (13) observed that α-keto butyric acid, ammonia, methanethiol, and dimethyl disulfide were produced by cell-free extract of *Pseudomonas* sp. Others reported production of keto butyric acid and methanethiol by cell extracts of *Pseudomonas* sp. (16), *Clostridium sporogenes* (27), and a soil bacterium (15). The production of α-aminobutyrate and methanethiol by cell extract of *Escherichia coli* was reported also (18).

In animal metabolism, the sulfur products of

dissimilation of methionine and cysteine are the same (7), and this is ascribed to transfer of the methionine sulfur to that of cysteine through cystathionine. Our experiments provided no evidence of such a conversion during dissimilation of methionine by the bacteria. Bacterium 14, which grew on both amino acids, released the cysteine sulfur as sulfate and the methionine sulfur as methanethiol and dimethyl disulfide. The fact that Garreau (9) detected sulfate as a product of methionine decomposition by *Aspergillus niger* suggests that cysteine is an intermediate product of this transformation.

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